Extraction, Localization, and Metabolism of Di-2-ethylhexyl Phthalate from PVC Plastic Medical Devices

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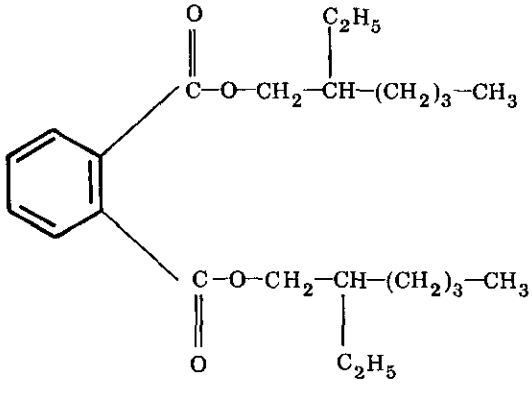
The results of our studies on the extraction and disposition of three plasticizing substances which are extracted from poly(vinyl chloride) medical devices will be reported. The plasticizers are di-2-ethylhexyl phthalate (DEHP), di-2-ethylhexyl adipate (DEHA), and butylglycolyl butyl phthalate (BGBP). DEHP extraction will be described in greater detail, and the two other plasticizers, DEHA and BGBP, will only be mentioned briefly.

The compound DEHP is an aromatic diacid ester that meets the physical criteria needed to soften and plasticize the rigid polymer poly(vinyl chloride) (PVC). As a plasticizer the material is added to PVC during manufacture and may represent between 20 and 40% of the finished weight of the plastic. The plasticizing agent is not firmly bound to the plastic but is in loose solution and, as such, may be extracted by a variety of organic solvents. Unfortunately, the plasticizer is to some extent soluble in biologic fluids such as blood and blood plasma. This is the key to this report.



Di-2-ethylhexyl phthalate (DEHP)

The present series of studies began with an unknown compound isolated from a liver perfusion system. This substance was characterized as a phthalate ester and was a metabolite of the plasticizer, BGBP. Subsequent investigation disclosed that yet another type of plastic tubing also resulted in contamination of the perfusate. The substance found in this case was not a metabolite, but the intact plasticizer. The results of an experiment with this type of tubing are shown in Figure 1. The tubing of the liver perfusion system was assembled, and perfusate (a blood and albumin solution) was pumped through the tubing in the absence of a liver. An amount of the same perfusion fluid was kept in the chamber at 37° C for the entire perfusion time. This was called noncirculated perfusion fluid. An organic extract of both perfusates was prepared, and analysis by thin-layer chromatography was performed. As can be seen, the sample of circulated perfusion fluid contains DEHP while the noncirculated perfusion fluid does not.

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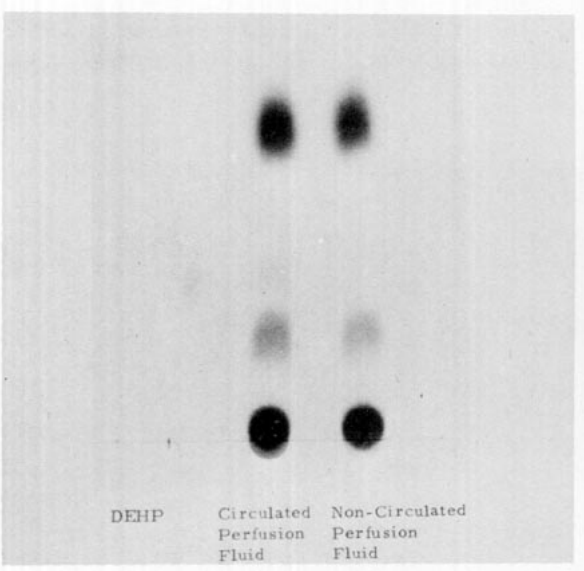

FIGURE 1. Thin-layer chromatogram of perfusion fluid *(1)*.

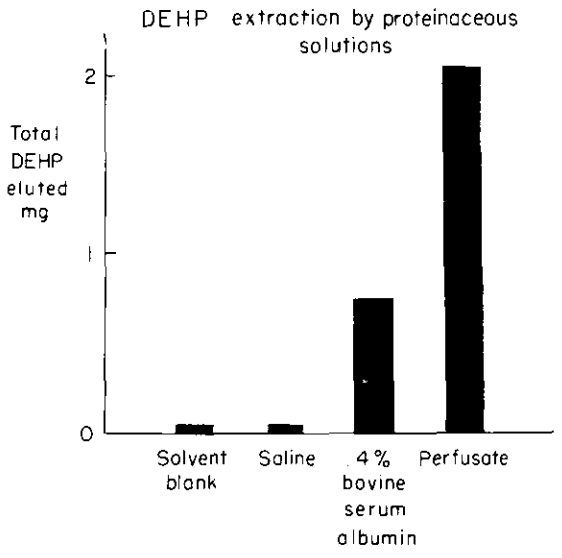

FIGURE 2. DEHP extraction by proteinaceous solutions *(1)*.

We attempted further to quantify the results obtained in this first experiment and also to determine what factor in perfusion fluid is responsible for DEHP extraction. Three separate perfusing solutions; saline, 4% bovine serum albumin (BSA) and perfusate, were pumped for 5 hr through the tubing used in an isolated perfusion system. The liver was omitted. It can be seen in Figure 2 that saline did not extract DEHP and was not different from the reagent blank. The 4% BSA solution, however, did extract 0.7 mg DEHP, while perfusate extracted almost three times as much, 2 mg DEHP. Thus, it appears that blood in the perfusion fluid with its additional content of cells, protein, and lipid is better able to extract DEHP than protein alone.

The observations that blood is capable of extracting the plasticizer from tubing in which it is transported led to the further question, whether human blood is capable of extracting DEHP from medical device tubing in which it is circulated. Specifically, medical grade poly(vinyl chloride) plastic tubing is employed in cardiopulmonary-bypass apparatus and in hemodialysis apparatus. Units of outdated blood (21 days old) were obtained from the Johns Hopkins Hospital and Baltimore City Hospital blood banks. The blood was circulated in the two types of medical device tubing for periods up to 8 hr. The results of these experiments are shown in Table 1. It can be seen that after 8 hr of circulation in the Travenol hemodialysis unit the DEHP content of the blood was 9.24 mg/100 ml. However, it is also striking that at zero time the DEHP concentration was 7.75 mg/100 ml. A similar result was obtained with cardiopulmonary bypass tubing: at 5 hr there was 5.7 mg/100 ml; at zero time the value was 5.04 mg/100 ml. The reason for this initial high concentration of DEHP is quite obvious: the units of blood were outdated and could no longer be used for transfusion because of age. For 21 days this blood had been stored at 4°C in

Table 1. Extraction of plasticizers from two types of medical devices.[a]

Medical device	Circulation time, hr	Plasticizer concentration, mg/100 ml blood DEHP	DEHA
Travenol hemodialysis unit	0	7.75	0
	8	9.24	2.14
Travenol cardiopulmonary bypass unit	0	5.04	0
	2.5	5.28	2.34
	5	5.70	4.99

[a]Data of Jaeger *(1)*.

PVC plastic blood bags. Thus, contamination of human blood occurred during storage. It is also interesting to note that another plasticizer, DEHA, was not present in the blood that came from the blood bank, but after circulation within the tubing. DEHA concentrations reached 2.14 and 4.99 mg/100 ml.

We attempted to establish the rate of DEHP extraction from plastic blood bags. It is more likely that blood might be contaminated during 21 day storage in plastic blood bags than it would be on circulation through plastic tubing. Therefore, units of human blood were obtained from the Johns Hopkins blood bank after 7, 11, 14, and 21 days of storage. The blood samples were analyzed for DEHP content. Samples of blood were also taken from units of dog blood which had been drawn and stored in a manner similar to that employed for human blood. The results of this experiment *(2)*, shown in Figure 3, indicate that the rate of DEHP accumulation in blood was 0.25 mg/100 ml/day over a period of 21 days of storage. It is also apparent that dog and human blood did not differ in their rates of extraction.

This result was examined further. A single unit, at 14 days of storage was divided into the following components: a triply washed red blood cell fraction and two plasma fractions, that portion of plasma with a buoyant density D greater than 1.21, and that fraction with a buoyant density of less than 1.21. As shown in Figure 4, there was very little DEHP in triply washed red cells. Approximately 20% of the DEHP was found in the high-density lipoprotein fraction, (D>1.21) but much more was contained in the low-density or buoyant lipoprotein fraction (D<1.21). This result suggests that the lipoproteins may solubilize DEHP. However, because the density of DEHP droplets is less than 1.21 (0.999), at room temperature, it is also possible that the fraction having density less than 1.21 represents DEHP which had floated to the surface. It is apparent, however, that the plasma fraction contains almost all of the DEHP that was found in whole blood.

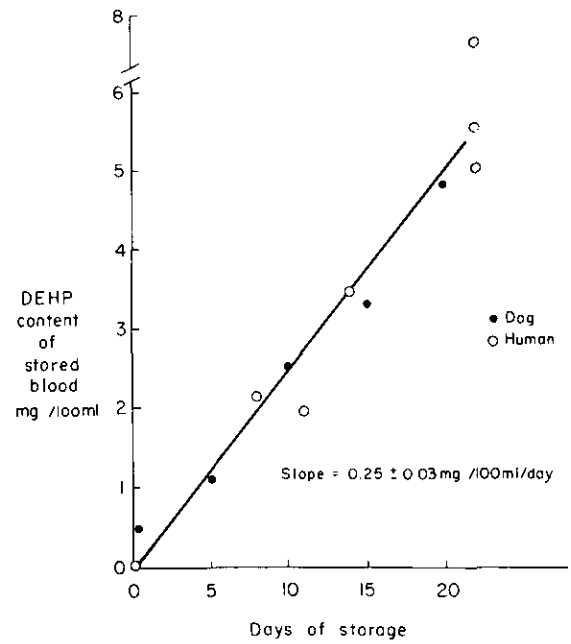

FIGURE 3. DEHP content of human and dog blood stored for varying periods in poly(vinyl chloride) blood bags *(2)*.

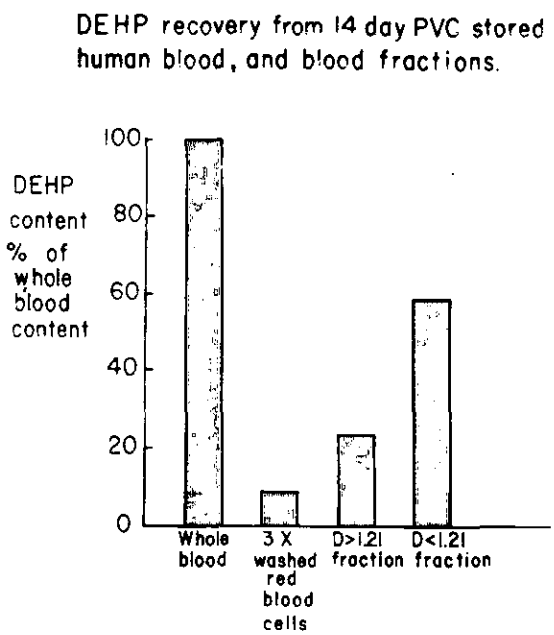

FIGURE 4. DEHP recovery from human blood and blood fractions stored 14 days in PVC *(1)*.

We carried our studies further and attempted to evaluate the amount of DEHP found in a fraction of whole blood, human platelet concentrates (Table 2). Five platelet concentrates were obtained from Johns Hop-

Table 2. DEHP in 10-ml aliquots of human platelet concentrate.[a]

Sample	DEHP, mg	Volume, ml	DEHP concentration, mg/100 ml
Platelet button			
1	0.196	0.6	32.7
2	0.185	0.5	37.0
3	0.220	0.5	44.0
4	0.191	0.4	47.8
5	0.189	0.7	27.0
	Mean ± S.E.M.		37.7 ± 3.8
	DEHP recovered		11.1%
Platelet-poor plasma			
1	1.688	9.3	18.2
2	2.227	9.5	23.4
3	1.613	9.5	17.0
4	1.447	9.5	15.2
5	0.881	9.2	9.6
	Mean ± S.E.M.		16.7 ± 2.2
	DEHP recovered		88.9%

[a]Data of Jaeger (*1*).

kins Hospital blood bank. The outdated units, stored for 48 hr at room temperature in ACD anticoagulant were kept in standard PL-130 plastic. We determined that in the five samples, the mean DEHP concentration in the unwashed platelet button was 37.7 ± 3.8 mg DEHP/100 ml packed platelet volume. This represented 11.1% of the total DEHP recovered from a 10-ml aliquot. The platelet-poor plasma was found to have a lower DEHP concentration than the platelet button, i.e., 16.7 ± 2.2 mg/100 ml. However, because of its much greater volume, the platelet-poor plasma contributed 88.9% of the total recovered DEHP. The rate of extraction of DEHP appears to be temperature-dependent. The concenctration achieved in platelet concentrates that had been stored at room temperature for 2 days was significantly greater than that ever reached in a unit of human blood stored at 4°C for 21 days.

We felt that another question should be asked: what becomes of DEHP which might subsequently be administered to a human? In our initial experiments, an acacia emulsion of DEHP was prepared and injected into the rat peritoneal cavity; 24 hr after injection of the emulsion the rat was sacrificed, and a midline incision made. Figure 5 shows the vicera of a rat after administration of the emulsion. The peritoneal cavity of this rat contained a great deal of ascitic fluid. Also, large white masses distributed throughout the peritoneal cavity were evident. Histologic examination indicated that the masses were serofibroid in nature, containing degenerating neutrophils and macrophages. The histopathology result suggested that the masses originated from a chemical peritonitis, possibly due to the administration of a material which was irritating to the peritoneal cavity. Control rats (given 3% acacia) were found to be unaffected by the vehicle.

To further clarify the fate of DEHP, emulsions were injected (IP) into rats which were then sacrificed at intervals after the injection. A whole-body homogenate was prepared, and aliquots sampled to determine the amount of DEHP which remained in the body after 2, 4, and 13 days. Figure 6 shows that the rate of DEHP disappearance after interperitoneal administration was quite slow. At 13 days, approximately 25% of the initial injected dose still remained. This fact,

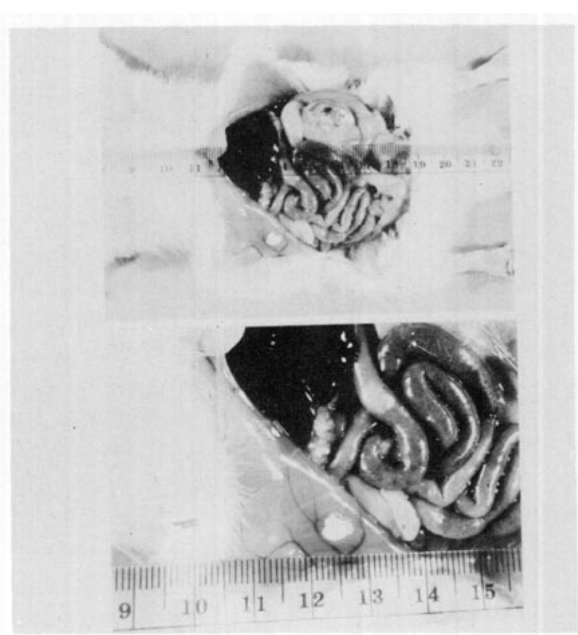

FIGURE 5. Gross pathology following intraperitoneal administration of DEHP (rat).

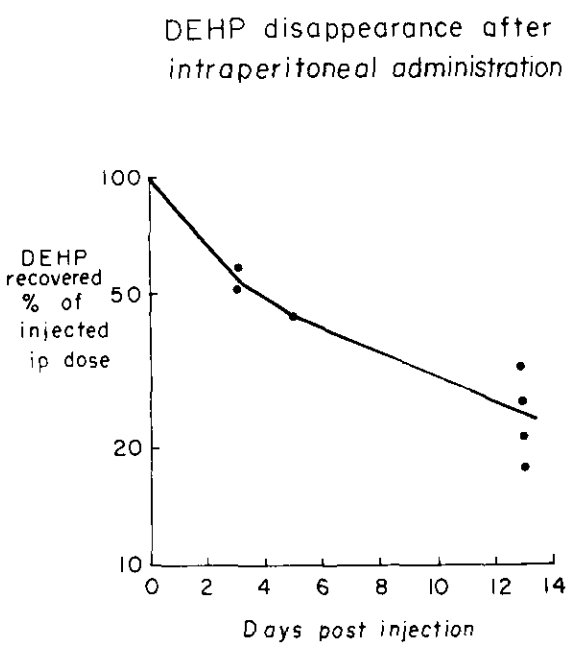

FIGURE 6. DEHP disappearance after intraperitoneal administration.

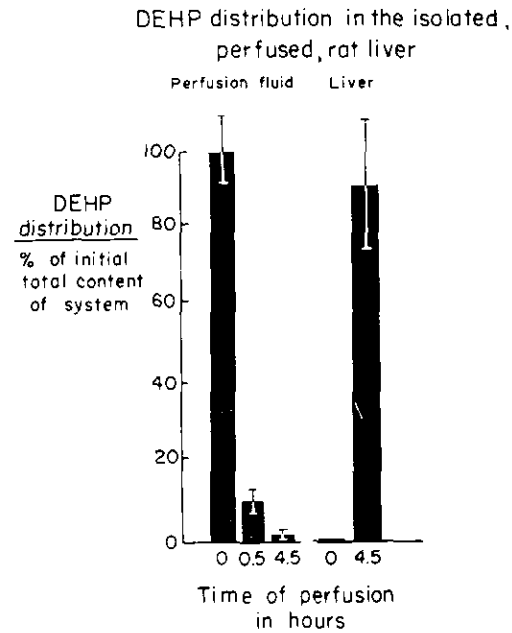

FIGURE 7. DEPH distribution in the isolated, perfused rat liver.

coupled with the rather marked peritoneal irritation and inflammation observed, might suggest that di-2-ethylhexyl phthalate is metabolically inert and irritating.

Because we found DEHP to be extracted by perfusion fluid in the isolated perfused-liver system we attempted to determine the fate of the DEHP in that system. We prepared perfusion fluid which contained 70–80 μg/ml DEHP and measured the total concentration of the system at zero time and after 0.5 and 4.5 hr. At the end of the perfusion the liver was removed, homogenized, and assayed for total DEHP content. The result (Fig. 7) indicates that within 30 min of perfusion, 90% of the total DEHP contained in the perfusate phase of the system has disappeared while in the remaining 4 hr an additional 9% was lost. At the end of 4.5 hr, the perfusate was essentially cleared of DEHP, but almost all the DEHP was recovered from the liver. This result is in marked contrast to our observations with BGBP, where we found a significant amount of metabolite in the perfusate. In the DEHP perfusion at 4.5 hr, no trace of phthalic acid was found. Phthalic acid would be the expected de-esterified metabolite of DEHP.

Because of the observation of accumulation by the isolated perfused liver system, we injected 40 mg of DEHP intravenously into two rats. The rats were sacrificed 24 hr later and tissue concentrations of DEHP determined. The results (Table 3) indicate that liver and lung contain the largest total amounts of the recovered dose. The carcass was also found to contain large amounts of DEHP but at a relatively low concentration. The spleen had a higher concentration, but only about 3% of the injected dose was found. The other tissues contained very little. The total amount of DEHP recovered was 79.1% of the injected dose. In similar experiments we were never able to find any trace of phthalic acid in the urine. This result might suggest that DEHP is inert in terms of the rat's ability to metabolize it to phthalic acid following IV injection. It is also possible that if DEHP is metabolized, it is converted to something other than phthalic acid *per se*.

If DEHP is extracted by blood from tubing and containers in which it is transported and stored, the possibility exists that this foreign compound may be administered to the human either when blood is admin-

Table 3. Intravenous DEHP rat tissue levels after 24 hr.[a]

Tissue	Concentration, mg/g or mg/ml	DEPH recovered from tissue, mg	% of injected dose recovered
Liver	1.6	21.82	27.3
Lung	8.18	21.26	26.6
Carcass	0.06	16.66	20.8
Spleen	1.89	2.65	3.3
Kidney	0.08	0.26	0.3
Heart	0.08	0.14	0.2
Gut	<0.01	0.12	0.2
Blood	<0.01	0.03	<0.01
Brain	<0.01	0.01	<0.01
Testicle	<0.01	0.01	<0.01
Feces	<0.01	0.334	0.4
		Total	79.1

[a] Data of Jaeger *(1)*.

istered, when open heart surgery is performed, or when hemodialysis occurs. Therefore, autopsy tissue from these categories of patients was sought and DEHP analysis performed. The results that were obtained *(2)* are shown in Table 4. It is evident from Table 4 that tissues from several persons who had exposure to PVC plastic do contain measurable amounts of DEHP. Notably, the first patient shown is an individual who received blood during cardiopulmonary bypass. The level of DEPH was 91.5 μg/g dry weight in the lung, 69.5 μg/g in the liver, and 25.3 μg/g in the spleen. However, during the bypass itself no DEHP was detectable in the systemic blood, a result which would suggest that some organ efficiently removes DEHP. Tissues from other patients sampled at autopsy similarly did contain DEHP but never at quite the concentration observed in the first patient. Conversely, several patients who had been exposed to DEHP were not found to have DEHP in their body tissues. One patient, an individual with a massive gunshot wound of the abdomen, had received 63 units of blood within a 24 hr period. From calculations involving use of the linear regression slope for DEHP extraction by blood at 4°C and its time of storage, it was calculated that this patient may have received 600 mg of the plasticizing agent. A sample of systemic blood taken at death was found to contain 0.28 mg DEHP/100 ml blood. Samples of lung and liver obtained at autopsy the following day did not contain any DEHP. In contrast to this, another patient in this study, a very obese female who had pancreatitis, had an extremely high concentration of DEHP in her abdominal fat. 270 μg/g. However this patient was an isolated case, and such a result was never duplicated. We did attempt to look for DEHP in abdominal fat of other individuals exposed to DEHP but did not find it. Also, 7 individuals (6 male, and 1 female) who died as a result of accidental causes and who were not known to have received transfusions did not have any DEHP in their abdominal fat. The results suggest that the amount of DEHP found in the female patient with pancreatitis was unique to her disease process or treatment history.

Because our results indicated that some but not all patients had measurable amounts of DEHP in their body tissues, we attempted to follow one person who was to undergo open heart surgery. This patient would presumably receive DEHP from transfusions or from exposure to PVC tubing. Samples of urine were obtained from the patient for 2 days before surgery and for 5 days following surgery. The urine was acidified, extracted with ether, and analyzed directly for phthalic acid. It was also treated by the method of Shaffer et al. *(3)*, i.e., the urine was hydrolyzed with acid and base in an attempt to determine whether some other form of phthalic acid was present. The results (Fig. 8) indicate that this was indeed the case. Prior to surgery, phthalic acid was present in the urine of this patient. However, in the period immediately following surgery, a total phthalate equivalent of 43 mg DEHP was calculated. We calculated that 23 mg resulted from known transfusions. The remaining 20 mg DEHP was probably received during the 1.5 hr bypass interval. It is apparent that not only is DEHP extracted from tubing by blood and transfused into human beings, but it does appear to be metabolized by the human. It is, however,

Table 4. DEHP content of human tissues.[a,b]

History no. or code (sex)	History	Units of blood adm'd	DEHP adm'd mg[c]	DEPH content, μg/g dry weight				DEPH in blood, mg/100 ml
				Lung	Liver	Spleen	Abdom. fat	
51 79 27 (F)	Card. pulm. bypass	18	43.8	91.5	69.5	25.3	–[d]	ND[e]
139 25 14 (M)	Card. pulm. bypass	4	14.0	24.5	—	—	—	—
117 67 25 (M)	Card. pulm. bypass	20	?[f]	22.1	—	—	—	—
139 93 59 (M)	Card. pulm. bypass	8	128.0	17.9	—	—	ND	—
130 84 99 (M)	Card. pulm. bypass	6	16.8	ND	ND	ND	—	—
138 60 69 (M)	Card. pulm. bypass	14	?	ND	ND	ND	ND	—
35 46 66 (M)	Aneurism	4	22.5	21.2	ND	5.0	—	—
138 89 87 (M)	Mult. transf. pancytopenia	?	?	20.8	—	—	ND	—
139 80 10 (M)	Mult. transf.	?	?	13.4	—	—	ND	—
44 66 84 (M)	GI bleeding	31	?	ND	ND	—	—	—
53 71 24 (F)	Pneumonia	2	?	ND	ND	ND	—	—
035 46 66 (F)	Pancreatitis	13	?	—	—	—	270.0	—
MEC (M)[g]	Gunshot	63	600.0	ND	ND	—	—	0.28
7 Indiv. (6M, 1F)	Accidental death	Not transfused		—	—	—	ND	—

[a]Data of Jaeger and Rubin (2).

[b]Assay blanks ranged from 20 to 60 μg DEHP. Reproducibility was ± 10%. Blank values were subtracted from tissue values for each analytical run and the net amount per gm of dry tissue is presented. Dry tissue weights ranged from 1.6 to 14.9 g.

[c]DEHP was calculated from the known storage time of each unit transfused and the previously determined DEHP migration rate of 0.25 mg/100 ml/day.

[d]Not assayed.

[e]Not detectable, i.e., not significantly different from the corresponding assay blank. This is equivalent to less than 0.5–3 μg/g.

[f]Number of units or storage time not known.

[g]Homocide victim, medical examiner case, Johns Hopkins history number not recorded.

metabolized to a form other than phthalic acid.

This latter point was studied further with three normal subjects; myself and two other graduate students, who donated urine samples for this study (Table 5). The urine, treated by the method of Shaffer et al., was found to contain amounts of phthalic acid, as did the urine from patient RM 2 days prior to surgery. This result would suggest that there are some phthalate compounds to which humans are exposed through environmental routes, possibly airborne inhalation of particles of plasticizers, its vapors, or by direct ingestion of phthalates from food.

In conclusion, these studies have shown that several plasticizers may be extracted from PVC tubing by human and animal blood. DEHP may be transfused into human beings by high concentrations, depending on the blood or its fraction administered. Further, some of the plasticizer, namely DEHP, does appear to accumulate in certain tissues and may be recovered at death from some, but not necessarily from all patients. At least one individual was capable of metabolizing the plasticizer, and normal persons had such substances in their urine. There is no information currently available which details exactly what toxicologic haz-

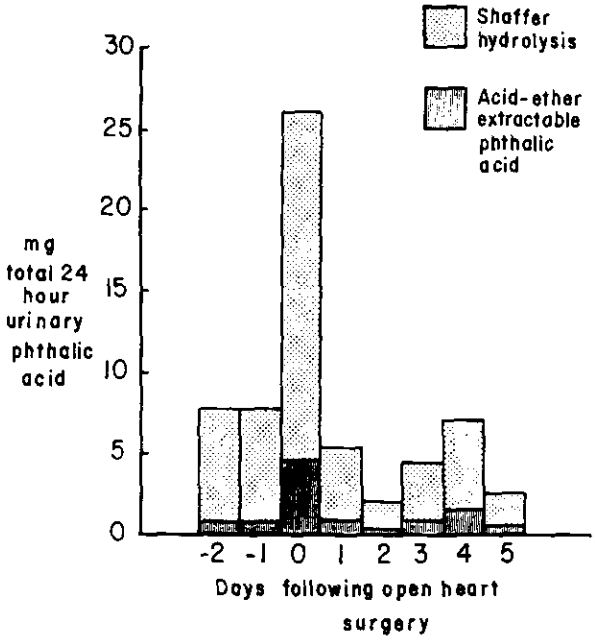

FIGURE 8. Urinary phthalate before and after open heart surgery.

ard may result from intravenous plasticizer exposure. Much information is conjectural, based on studies in animals who are normally healthy and not stressed. It is our recommendation that poly(vinyl chloride) plastic formulations be developed that do not contribute any extractable material. Alternatively, plastic formulations should be found which do not contain any additive and as such, will not contribute any material to blood or its fractions that are stored or transported in them.

Table 5. Hydrolysis by the Method of Shaffer et al. *(3)* for urinary "phthalic acid" content of *normal individuals.*

Subject		Concentration, mg PA/100 ml
Patient	RM	0.48
	RJJ	0.36
	JK	0.74
	JH	1.29

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